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Abstract

Anodal direct currents at intensities ranging from 0.3 to 30.0 microA were unilaterally applied for 30 min once a day to the premotor area of the rabbit cerebral cortex. The anodal polarization was repeated 10 times at intervals of 2-3 days, and the effect on the motor activity of the forelimbs during and after each polarization trial was compared with that before polarization. Peripheral motor activity was classified as either gentle flexion of forelimbs or struggle with violent movement of forelimbs. A current of 0.3 microA caused no change in motor behavior. Flexion of the forelimb contralateral to the polarized cortex was clearly increased when a polarizing current of 1.0 or 3.0 microA was applied, and peak flexion was observed between the third and seventh polarization trials. A current of 10 or 30 microA had no effect on forelimb flexion. Conversely, forelimb struggle on both sides was decreased when 10.0 or 30.0 microA, but not 1.0 or 3.0 microA, was applied. These results show that anodal polarization of the cerebral cortex exerts dual effects on peripheral motor activity, probably through changes in cortical excitability associated with the current intensity.

KEYWORDS: anodal polarization, dominant focus, motor behavior, cerebral cortex, rabbit

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Anodal direct currents at intensities ranging from 0.3 to 30.0 μA were unilaterally applied for 30 min once a day to the premotor area of the rabbit cerebral cortex. The anodal polarization was repeated 10 times at intervals of 2-3 days, and the effect on the motor activity of the forelimbs during and after each polarization trial was compared with that before polarization. Peripheral motor activity was classified as either gentle flexion of forelimbs or struggle with violent movement of forelimbs. A current of 0.3 μA caused no change in motor behavior. Flexion of the forelimb contralateral to the polarized cortex was clearly increased when a polarizing current of 1.0 or 3.0 μA was applied, and peak flexion was observed between the third and seventh polarization trials. A current of 10 or 30 μA had no effect on forelimb flexion. Conversely, forelimb struggle on both sides was decreased when 10.0 or 30.0 μA, but not 1.0 or 3.0 μA, was applied. These results show that anodal polarization of the cerebral cortex exerts dual effects on peripheral motor activity, probably through changes in cortical excitability associated with the current intensity.

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Prolonged application of a weak anodal direct current to the surface of the cerebral cortex has been shown to increase neuronal firing in several species of animals (1-3), and is usually accompanied by characteristic peripheral motor manifestations including forelimb flexion (4-6). The peripheral phenomenon is suggested to be due to neuronal hyperexcitability, the so-called dominant focus, in the polarized cortex, which is possibly induced by anodal polarization (4). The behavioral effects of anodal polarization persist for several hours (7) to weeks (5, 8) after current application. Thus, the dominant focus, a chronic excitation focus, in the cortex is a useful model for understanding the mechanisms of central plastic changes, such as memory, learning, and conditioning reflex (1, 2, 9, 10).

In earlier studies with the rabbit (4, 5, 7, 8), attempts were made to determine the optimal polarization conditions for inducing the dominant focus, in which the frequency of forelimb flexion during and after polarization was used as an indicator of cortical activity enhancement. Based on motor manifestation results thus far obtained, it is suggested that the intensity and duration of polarizing currents and the frequency with which they are applied are crucial factors for inducing the dominant focus. While much attention has been paid to the attainment of target motor behavior, no general characteristics of anodal polarization have been described due to the complicated nature of the models used to date. In the present study, simplified experimental schedules for anodal polarization with a wide range of current intensities were used to determine the optimal experimental conditions for inducing the motor dominant focus resulting in peripheral motor activity in the rabbit, in which flexion and struggle of the forelimbs were observed and analyzed systematically in relation to the polarization model.

Materials and Methods

Animals and surgery. Male albino rabbits weighing 2.7-3.2 kg were used in this study. Rabbits were anesthetized with an intravenous injection of sodium pentobarbital (40 mg/kg) and placed on a stereotaxic apparatus. The cranial bone was exposed, and trephine hollows were made bilaterally in the bone over the premotor cortex at a point 3 mm lateral and 7 mm rostral to the bregma. Two silver electrodes (1 mm in diameter)

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were implanted in the hollows so as to set the electrode tip in the bone. The electrodes were connected to the pins of a miniature socket and secured to the skull with a dental resin. Rabbits were allowed to recover from surgery for more than 10 days before the start of experiments.

Anodal polarization. Rabbits were restrained in a wooden pillory on the experimental stand in the prone position. The hindlimbs were tightly fastened to the stand with cloth belts. The forelimbs were bound at the wrist with cloth belts and slightly stretched forward to allow movement, and were connected to a mechanograph to record flexion and struggle movements.

Before polarization, rabbits were restrained for 60 min on the experimental stand to allow habituation to the experimental conditions. Habituation was repeated once a day for 5 days. No current was delivered during the habituation periods. For polarization, direct current from the anode of a dry battery was passed through the electrode to the left premotor cortex, which has been reported to be appropriate for establishing the target motor behavior (5, 8). A silver plate attached to the left pinna was connected to the cathode as a counter electrode. Rabbits were randomly divided into five groups, and a constant current of 0.3, 1.0, 3.0, 10.0 or 30.0 μA was applied to each group. The duration of current application was 30 min. Polarization was performed once a day. The output intensity of the currents was monitored with a microammeter during polarization. To stabilize the polarizing current, a 1–5 MΩ resistor was connected to the polarizing circuit in series. Furthermore, the current was initially switched on with a minimal intensity and then tuned to the prescribed intensity to avoid an initial surge. The current was tapered off at the end of polarization to avoid a sudden opposite directional gradient of current.

The 30-min anodal polarization was repeated 10 times at intervals of 2–3 days. The experiments were completed within 6 weeks after the beginning of habituation.

Determination of behavioral activity. The behavioral activity was determined in the habituation period (60 min, 5 times), and during (30 min) and after (60 min) each polarization trial. The forelimb movements were classified as flexion and struggle. The flexion was gentle, slow, and smooth movements of either or both forelimbs with no associated change in posture. The struggle was violent, quick, and strong movements of both forelimbs which were usually accompanied by whole body movements.

The forelimb movements, flexion and struggle, were recorded with a mechanograph, and also a videocamera for further analysis. The flexion of the left and right forelimbs appeared as single or multiple episodes. Left and right forelimb flexions were evaluated separately and counted as one episode of flexion when the forelimb was lifted up and put back. Struggle appeared as a cluster of highly frequent movements with no difference between the left and right forelimbs. The cluster of forelimb movements was counted as one episode of struggle.

Statistical analysis. Data are expressed as means or means $\pm$SEM. For comparison, the number of flexions or struggles during (30 min) and after (60 min) polarization were accumulated in 10 trials, in which the values after polarization were converted to those per 5 h. Statistical significance was evaluated by the two-tailed Mann-Whitney *U*-test and values of $P < 0.05$ were taken to indicate significance. Values obtained for a 5-h habituation period were used as the control.

Results

Increase in forelimb flexion by anodal polarization. The mean value of flexion of the left and right forelimbs throughout the experimental schedule is shown in Fig. 1. The flexions of the forelimb contralateral to the polarized cortex were significantly increased by anodal polarization with 3.0 μA, as compared with those in the habituation (control) period; increased flexion activity was detected both during and after polarization (Fig. 1B). A significant increase was also observed with 1.0 μA, although to a lesser extent than 3.0 μA (Fig. 1B). With application of the other current intensities (0.3, 10.0 and 30.0 μA), there was a tendency for the flexion activity to increase, but this was not statistically significant. Ipsilateral forelimb flexion did not increase significantly with any current intensity (Fig. 1A).

Time-dependent change in polarization-induced forelimb flexion. The time course of forelimb flexions throughout 10 polarization trials is shown in Figs. 2–4. Contralateral forelimb flexion abruptly increased during the fifth trial of anodal polarization with 1.0 μA, and thereafter flexions decreased gradually (Fig. 2A). In the fifth trial, the increased flexion activity lasted for at least 60 min after polarization (Fig. 2B). Such a time-dependent increase in flexion activity was detected during the fifth and seventh trials, and after the third trial of polarization with 3.0 μA (Fig. 3A, B). No apparent time-dependent change in forelimb flexions was observed with 10.0μA (Fig. 4A, B) or with the other intensities tested (data not shown).

Decrease in forelimb struggle by anodal polarization. The mean value of forelimb struggles throughout the experiment is shown in Fig. 5. Forelimb struggles were significantly decreased by anodal polarization with 10.0μA, as compared with those in the control period; the decreased struggle activity was detected both during and after polarization. Such a significant decrease in struggle activity was also observed with 30.0μA. The forelimb struggle did not change significantly with any other current intensity.

Time-dependent change in polarization-induced forelimb struggle. The time course of forelimb struggles throughout 10 polarization trials is shown in Fig. 6. Forelimb struggles decreased during the second trial of anodal polarization with 10.0μA (Fig. 6A), and decreased struggle activity was also observed after polarization (Fig. 6B). Such a time-dependent decrease in struggle activity was also detected with 30.0μA. With both 10.0 and 30.0μA, struggle activity remained low throughout the polarization trials. No apparent time-dependent changes in forelimb struggle were observed with 1.0μA (Fig. 6A, B) or with the other intensities tested (data not shown).

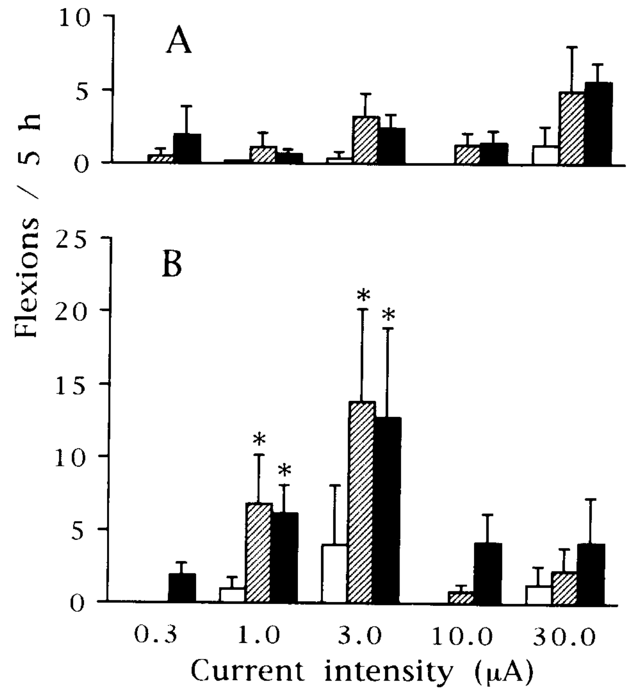

Fig. 1 Effects of anodal polarization on flexions of the left and right forelimbs in rabbits. Polarizing currents of 0.3, 1.0, 3.0, 10.0, and 30.0μA were applied for 30 min to the left premotor cortical area. The 30-min anodal polarization was repeated 10 times once a day at intervals of 2–3 days. The number of flexions of the forelimb ipsilateral (**A**) and contralateral (**B**) to the polarization was determined before polarization (habituation period, open columns), during (hatched columns), and after (solid columns) each trial of anodal polarization. Values are the means ±SEM of the number of flexions per 5 h for 3–7 different rabbits. *Significant difference from the control value before polarization, $P < 0.05$.

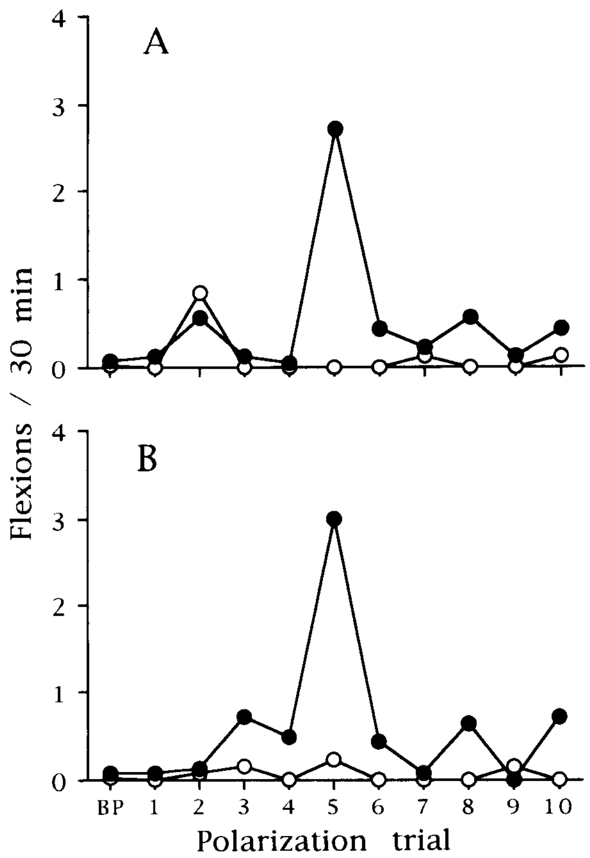

Fig. 2 Time course of effects of anodal polarization with 1.0μA on forelimb flexions in rabbits. Polarizing current of 1.0μA was applied for 30 min and it was repeated 10 times. During (**A**) and after (**B**) each polarization trial, the flexions of the left (open circles) and right (solid circles) forelimbs were determined. BP indicates the control value before polarization. Values are the means of the number of flexions per 30 min for 7 different rabbits.

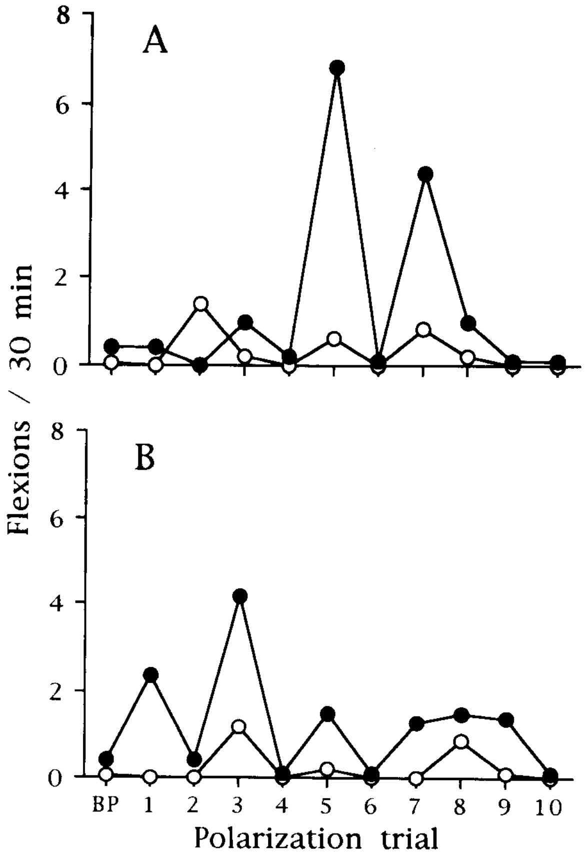

Fig. 3 Time course of effects of anodal polarization with 3.0 μA on forelimb flexions in rabbits. Polarizing current of 3.0 μA was applied for 30 min and it was repeated 10 times. The left and right forelimb flexions were determined during (**A**) and after (**B**) each polarization trial. Symbols are the same as in Fig. 2. Values are the means of the number of flexions per 30 min for 5 different rabbits.

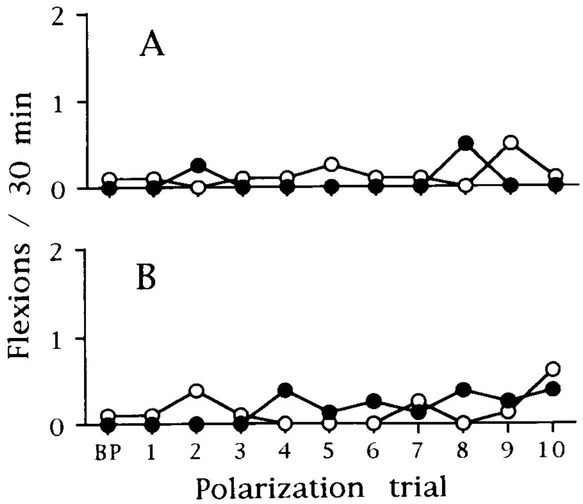

Fig. 4 Time course of effects of anodal polarization with 10.0 μA on forelimb flexions in rabbits. Polarizing current of 10.0 μA was applied for 30 min and it was repeated 10 times. The left and right forelimb flexions were determined during (**A**) and after (**B**) each polarization trial. Symbols are the same as in Fig. 2. Values are the means of the number of flexions per 30 min for 4 different rabbits.

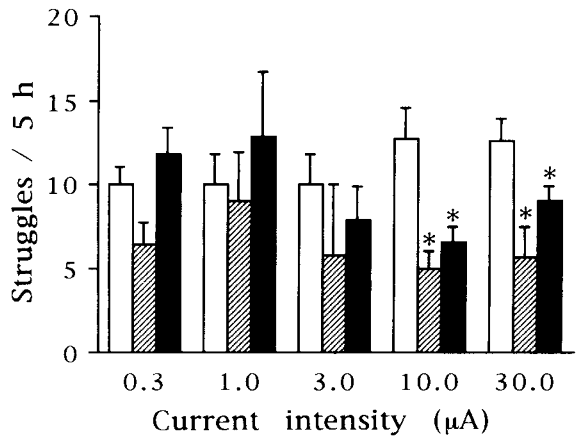

Fig. 5 Effects of anodal polarization on struggles in rabbits. The 30-min anodal polarization with 0.3, 1.0, 3.0, 10.0, and 30.0 μA was repeated 10 times. The number of struggles was determined before polarization (habituation period, open columns), during (hatched columns), and after (solid columns) each polarization trial. Values are the means $\pm$ SEM of the number of struggles per 5 h for 3-7 different rabbits. *Significant difference from the control value before polarization, $P < 0.05$.

Discussion

Previous studies have shown that repeated anodal polarizations of the unilateral cerebral cortex increase the flexion of the contralateral forelimb in the rabbit (4-6, 8), although the optimal current intensity has not yet been found because of wide variations in experimental conditions. The present results, obtained using simplified polarization schedules, clearly show that contralateral forelimb flexion is increased by repeated polarizations of the premotor cortex of rabbits with 1.0 and 3.0 μA, but not with 0.3, 10.0, or 30.0 μA. The results indicate that anodal polarization at current intensities ranging from 1.0

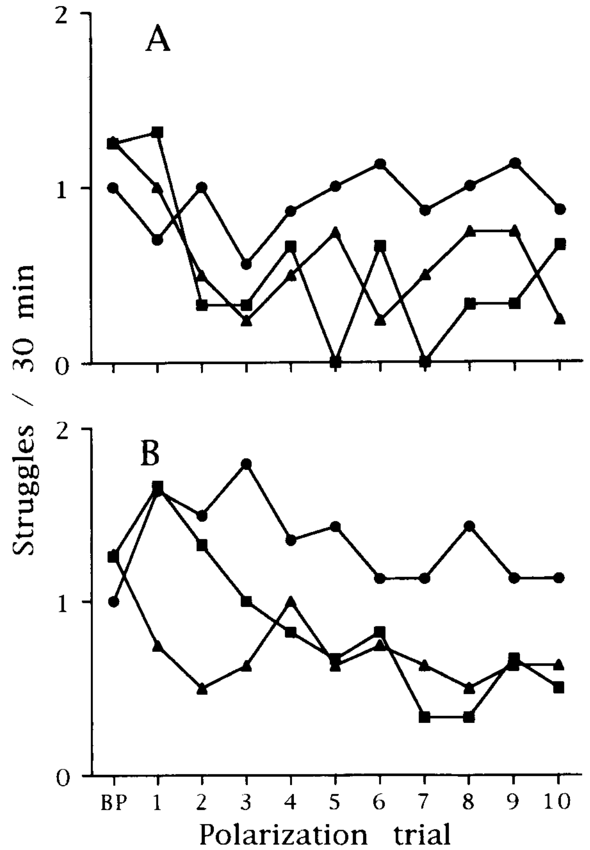

Fig. 6 Time course of effects of anodal polarization on struggles in rabbits. The 30-min anodal polarization with 1.0, 10.0, and 30.0 μA was repeated 10 times. The number of struggles was determined during (A) and after (B) each trial of polarizations with 10.0 μA (triangles), 30.0 μA (squares), and 1.0 μA (circles). Values are the means of the number of struggles per 30 min for 3-7 different rabbits.

to 3.0 μA was most appropriate for establishing the target motor manifestation of the forelimbs under the present experimental conditions. Further, the results of this study also indicate that the forelimb flexion was at a peak level between the third and seventh polarization trials even when the optimal current intensity was used. Thus, it is likely that the optimal model for anodal polarization, including both the current intensity and frequency of application, is necessary for establishing the target motor behavior, in which the forelimb flexions may progressively increase through possible cumulative effects of anodal polarizations.

Concerning the mechanism of anodal polarization-induced central plastic changes, cortical polarization has been shown to increase neuronal firing in the rat and cat (1, 2, 11). In addition, Gartside (9) suggested that synaptic modulation in the polarized cortical area was associated with increased neuronal activity. Rusinova (12) reported that an increase in electroencephalographic coherence between the sensomotor cortex and the dorsal hippocampus was noted when the motor dominant focus was induced by anodal polarization. In this context, our recent studies with the cortical tissue of polarized cortex (13-15) have revealed that cyclic AMP generation elicited by noradrenaline or adenosine is increased or decreased by anodal polarization, and this effect is associated with the duration and intensity of polarization. It has also been reported that both protein synthesis and neuronal firing are increased by anodal polarization (16) and the increase in neuronal activity is suppressed by pretreatment with an inhibitor of protein synthesis (17). These findings suggest that anodal polarization affects the efficiency of synaptic transmission as well as the neuronal firing, partly through changes in cyclic AMP generation and protein synthesis in the central nervous system. It seems reasonable to surmise that the occurrence of central plastic change requires several days; this aspect is evidenced by the fact that the repetition of anodal polarization is essential for establishing the peripheral motor activity even at the optimal current intensity.

The concept of struggles was introduced into this study to allow systematic analysis of the data; the results indicate that struggle was decreased by anodal polarization with 10.0 and 30.0 μA, during and after which rabbits were silent and drowsy. However, the polarizing currents of 0.3, 1.0, or 3.0 μA had no apparent effect on struggle. These results suggest that the polarizing currents have an inhibitory effect on cortical activity resulting in behavioral sedation, in addition to their excitatory effect. The excitatory and inhibitory effects of polarizing currents are supported by the following findings: Neuronal activity in rat cerebral cortex was enhanced by anodal polarization at a certain current intensity, whereas it was suppressed by stronger currents or longer polarization (1, 2, 11). Similar effects were also noted in the amplitude of somatosensory evoked potential (2) and also in cyclic AMP generation in the polarized cortex (14). Taken together, the results of this study provide evidence for the idea that anodal polarization exerts dual effects on peripheral motor activity, probably through changes in cortical excitability associated with the current intensity.

In conclusion, the repetition of unilateral anodal polarization of the premotor area of the rabbit cerebral cortex with 1.0 and 3.0 μA clearly promotes flexion of contralateral forelimbs. This effect was most pronounced between the third and seventh polarizing trials. Polarization with 10.0 and 30.0 μA resulted in a decrease in struggles from the second trial onwards. These results suggest that anodal polarization exerts dual effects on peripheral motor activity: a stimulatory effect in a current intensity range of 1.0 to 3.0 μA and sedative effect with stronger currents. The dual effects of polarizing currents may be due to changes in cortical excitability relative to their intensity.

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